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CLAIMS

1. A plasmid for use in monitoring the efficiency of a restriction endonuclease digestion, comprising:

(a) at least one spacer segment comprising a nucleic acid sequence that is restriction site-free; and

(b) at least two polylinker regions containing a plurality of unique restriction sites distributed so that digestion of the plasmid with any two restriction endonucleases whose sites are represented on said plasmid results in two fragments, said fragments being sufficiently different in size from the intact plasmid so as to be readily distinguishable from said plasmid.

2. The plasmid of claim 1 wherein the size of one of said fragments is at least about 15% less than the intact plasmid.

3. A plasmid for use in monitoring the efficiency of a restriction endonuclease digestion, comprising:

(a) at least one spacer segment comprising a nucleic acid sequence that is restriction site-free; and

(b) at least two polylinker regions wherein said polylinker regions contain a plurality of unique restriction sites distributed so that, for any two sites, the two sites are situated within different polylinkers on said plasmid and wherein said polylinker regions are separated by a spacer segment whose length is about 15-85% of the length of the plasmid.

4. The plasmid of claim 1, wherein the length of said spacer segment is about 20-85% of the length of the plasmid.

5. The plasmid of claim 1, wherein the length of said spacer segment is about 30-85% of the length of the plasmid.

6. The plasmid of claim 1, wherein the length of said spacer segment is about 40-85% of the length of the plasmid.

7. The plasmid of claim 1, wherein the length of said spacer segment is about 50-85% of the length of the plasmid.

8. The plasmid of claim 1, wherein digestion of said plasmid with two endonucleases whose recognition sites are represented on said plasmid results in two fragments, one of said fragments being at least about 15% of the length of the undigested plasmid.

9. The plasmid of claim 1 further comprising a replication origin and a selectable marker.

10. The plasmid of claim 1 further comprising a vector backbone of a plasmid selected from the group consisting of pUC, pBR322 and pBS.

11. The plasmid of claim 1 wherein said plasmid is linearized prior to use in an endonuclease digestion reaction.

12. A set of plasmids for use in monitoring the efficiency of a restriction endonuclease digestion, wherein each of said plasmids comprises:

(a) at least one spacer segment comprising a nucleic acid sequence that is restriction site-free; and

(b) at least two polylinker regions wherein said polylinker regions contain a plurality of unique restriction sites distributed so that, for any two sites, the two sites are situated within different polylinker regions on at least one plasmid of said set and wherein said polylinker regions are separated by a spacer segment whose length is about 15-85% of the length of the plasmid.

13. The set of claim 12, wherein the length of said spacer segment is about 20-80% of the length of the plasmid.

14. The set of claim 12, wherein the length of said spacer segment is about 30-80% of the length of the plasmid.

15. The set of claim 12, wherein the length of said spacer segment is about 40-80% of the length of the plasmid.

16. The set of claim 12, wherein the length of said spacer segment is about 50-80% of the length of the plasmid.

17. A method for designing a plasmid for use in monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) identifying at least one spacer segment comprising a nucleic acid sequence that is restriction site-free;

(b) identifying a plurality of restriction sites to be represented on said plasmid;

(c) assigning each of said restriction sites to a polylinker region on said plasmid such that for any two restriction sites, the two sites are situated in different polylinkers;

(d) distributing said polylinker regions on said plasmid such that said polylinker regions are separated by a spacer segment at least about 15%-85% of the length of the plasmid.

18. The method of claim 17 wherein digestion of said plasmid with any two endonucleases represented on said plasmid results in two fragments, one of said fragments being at least about 15%-85% of the length of the intact plasmid.

19. A method for designing a set of plasmids for use in monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) identifying at least one spacer segment comprising a nucleic acid sequence that is restriction site-free;

(b) identifying a plurality of restriction sites to be represented on said plasmids;

(c) assigning each of said restriction sites to a polylinker region on one of said plasmids such that for any two restriction sites, there is at least one plasmid in the set in which the two sites are situated in different polylinkers;

(d) distributing said polylinker regions on said plasmids such that said polylinker regions are separated by a spacer segment at least about 15%-85% of the length of the plasmid.

20. A method for designing a set of plasmids for monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) identifying at least one spacer segment comprising a nucleic acid sequence that is restriction site-free;

(b) identifying a plurality of restriction sites to be represented on said plasmids;

(c) determining the number of polylinker regions (*a*) that will accommodate said restriction sites, wherein the maximum number (N) of sites which can be represented is $N = a^b$, where *a* is the number of polylinkers in each plasmid, *b* is the number of plasmids in the set; and

(e) assigning each of said restriction sites to a polylinker region in accordance with a template, wherein said template corresponds to an *a* x *b* matrix, and wherein each of said sites is in a different polylinker from any of the other sites in at least one of the plasmids in the set.

21. A set of plasmids constructed according to the method of claim 20.

22. The set of plasmids of claim 21 further comprising additional restriction sites situated in vector cloning sites of the plasmids.

23. A method for designing a set of three plasmids for monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) identifying at least one spacer segment comprising a nucleic acid sequence that is restriction site-free;

(b) identifying 27 restriction sites to be represented on said plasmids;

(c) numerically ordering said restriction sites;

(d) assigning each of said restriction sites to a polylinker region, wherein

(i) sites 1-9 are assigned to a first polylinker on a first plasmid, sites 10-18 are assigned to a second polylinker on said first plasmid and sites 19-27 are assigned to a third polylinker on said first plasmid;

(ii) sites 1, 4, 7, 10, 13, 16, 19, 22 and 25 are assigned to a first polylinker on a second plasmid, sites 2, 5, 8, 11, 14, 17, 20, 23, and 26 are assigned to a second polylinker on said second plasmid and sites 3, 6, 9, 12, 15, 18, 21, 24, and 27 are assigned to a third polylinker on said second plasmid;

(iii) sites 1, 2, 3, 10, 11, 12, 19, 20 and 21 are assigned to a first polylinker on a third plasmid, sites 4, 5, 6, 13, 14, 15, 22, 23 and 24 are assigned to a second polylinker on said third plasmid and sites 7, 8, 9, 16, 17, 18, 25, 26 and 27 are assigned to a third polylinker on said third plasmid; and

(e) distributing said polylinker regions on each of said plasmids such that said polylinker regions are separated by a spacer segment at least about 15% of the length of the plasmid.

24. A set of plasmids constructed according to the method of claim 23.

25. The set of plasmids of claim 24 further comprising additional restriction sites situated in vector cloning sites of the plasmids.

26. A method of constructing a set of four plasmids for monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) identifying at least one nucleic acid sequence that is restriction site-free;

(b) identifying 64 restriction sites to be represented on said plasmids;

(c) numerically ordering said restriction sites;

(d) assigning each of said restriction sites to a polylinker region, wherein

(i) sites 1-16 are assigned to a first polylinker on a first plasmid, sites 17-32 are assigned to a second polylinker on said first plasmid, sites 33-48 are assigned to a third polylinker on said first plasmid and sites 49-64 are assigned to a fourth polylinker on said first plasmid;

(ii) sites 1-4, 29-32, 41-44 and 53-56 are assigned to a first polylinker on a second plasmid, sites 5-8, 17-20, 45-48 and 57-60 are assigned to a second polylinker on said second plasmid, sites 9-12,21-24, 33-36 and 61-64 are assigned to a third polylinker on said second plasmid and sites 13-16, 25-28, 37-40 and 49-52 are assigned to a fourth polylinker on said second plasmid;

(iii) every fourth site beginning with site number 1 is assigned to a first polylinker on a third plasmid, every fourth site beginning with site number 2 is assigned to a second polylinker on said third plasmid, every fourth site beginning with site number 3 is assigned to a third polylinker on said third plasmid; and every fourth site beginning with site number 4 is assigned to a fourth polylinker on said fourth plasmid.

(e) distributing said polylinker regions on each of said plasmids such that said polylinker regions are separated by a restriction site-free region at least about 15% of the length of the plasmid.

27. A set of plasmids constructed according to the method of claim 26.

28. The set of plasmids of claim 27 further comprising additional restriction sites situated in vector cloning sites of the plasmid.

29. A method for producing plasmids for monitoring the efficiency of a restriction endonuclease digestion, comprising the steps of:

(a) transfecting host cells with the plasmid of claim1;

(b) growing said cells under conditions to provide a quantity of cells containing said plasmids; and

(c) purifying said plasmids from said cells.

30. A method for monitoring the efficiency of a restriction endonuclease digestion comprising:

(a) adding a monitor plasmid of claim 1 to a sample preparation of non-monitor plasmids;

(b) initiating an endonuclease digestion reaction;

(c) allowing the reaction to proceed to completion;

(d) determining whether the monitor plasmid has been digested, wherein digestion of said monitor plasmid indicates digestion of non-monitor plasmids in said sample preparation.

31. The method of claim 30 wherein determining whether the monitor plasmid has been digested is by visualization of products of the endonuclease digestion.

32. The method of claim 30, wherein visualization of said products is by gel electrophoresis.

33. A kit for monitoring the digestion efficiency of a restriction endonuclease digestion reaction comprising:

(a) at least one plasmid wherein said plasmid contains a plurality of unique restriction sites distributed so that digestion of said plasmid with any two restriction endonucleases represented on the plasmid results in two plasmid fragments, one of said fragments being at least about 15% of the length of the undigested plasmid; and

(b) instructions for use of said plasmid.

34. The kit of claim 33, wherein the kit comprises one plasmid.

35. The kit of claim 33, wherein the kit comprises two plasmids.

36. The kit of claim 33, wherein the kit comprises three plasmids.

37. The kit of claim 33, wherein the kit comprises four or more plasmids.

38. The kit of claim 33, further comprising restriction endonucleases for sites represented on said plasmids.

39. The kit of claim 38, further comprising appropriate buffers for the restriction endonucleases represented on said plasmids.

40. The set of claim 12, as shown in FIGURE 1.

41. The set of claim 12, as shown in FIGURE 2.

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